Nucleotides as anti-HBV agents

Radhakrishnan P. Iyer, Ph.D.

Spring Bank Technologies, Inc.,

113 Cedar Street, Milford, MA 01757, USA.
kiyer@springbanktech.com

Challenges in Antiviral Drug Discovery

Viruses cause life-threatening illnesses worldwide

Discovery Issues

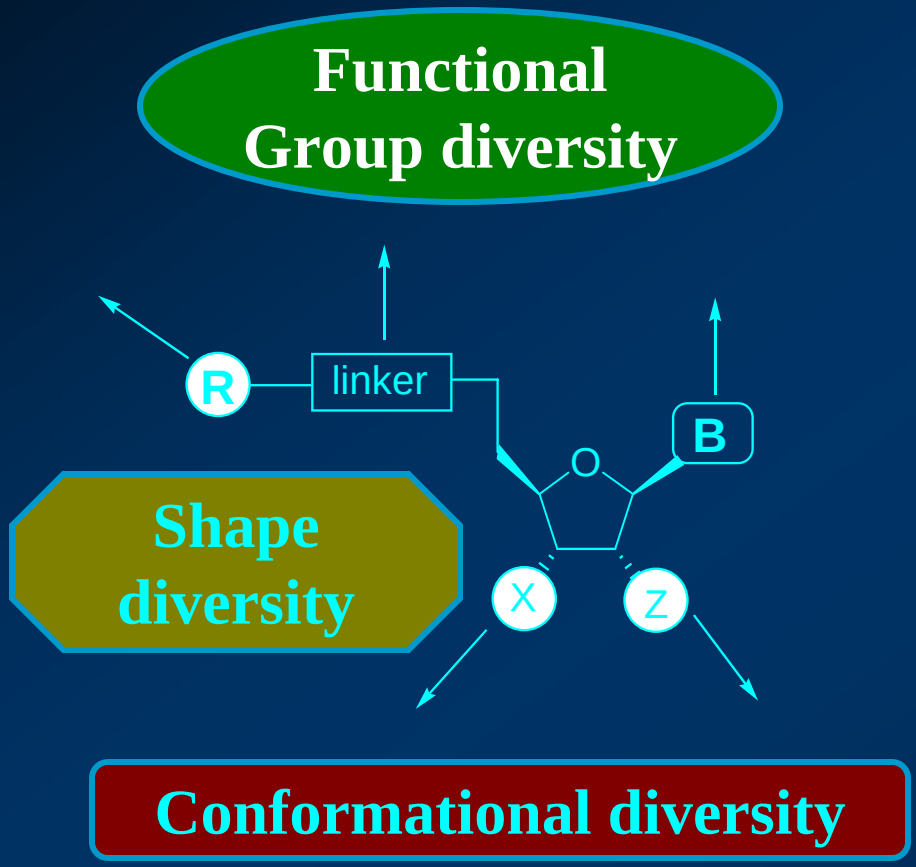
- Lack of druggable targets
- Emergence of resistant mutants

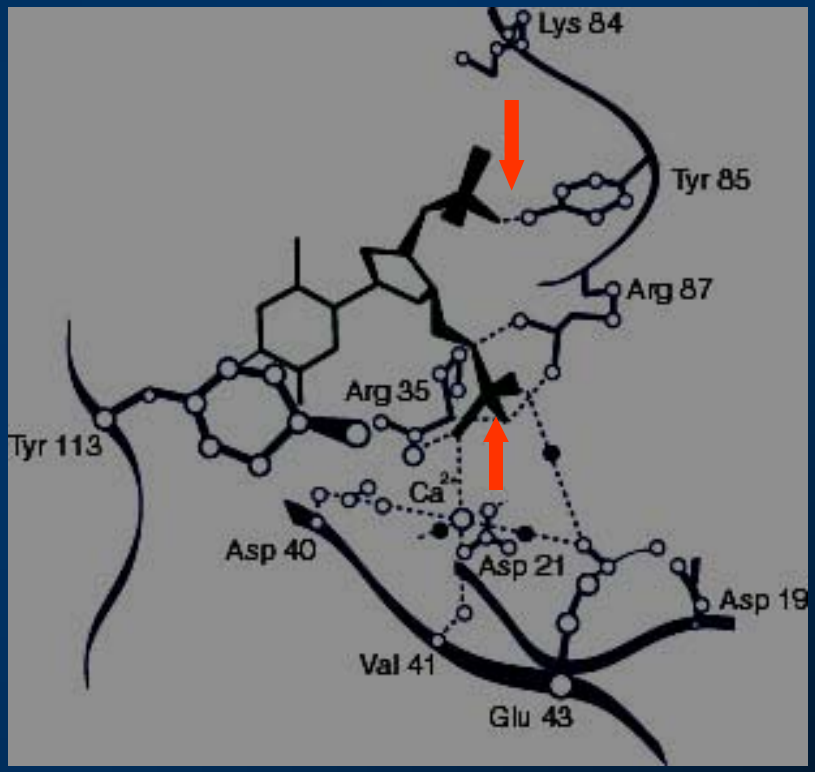
Therapeutic Issues

- Rapid emergence of antiviral resistance
- Dose-limiting toxicity

Spring Bank Technologies, Inc.

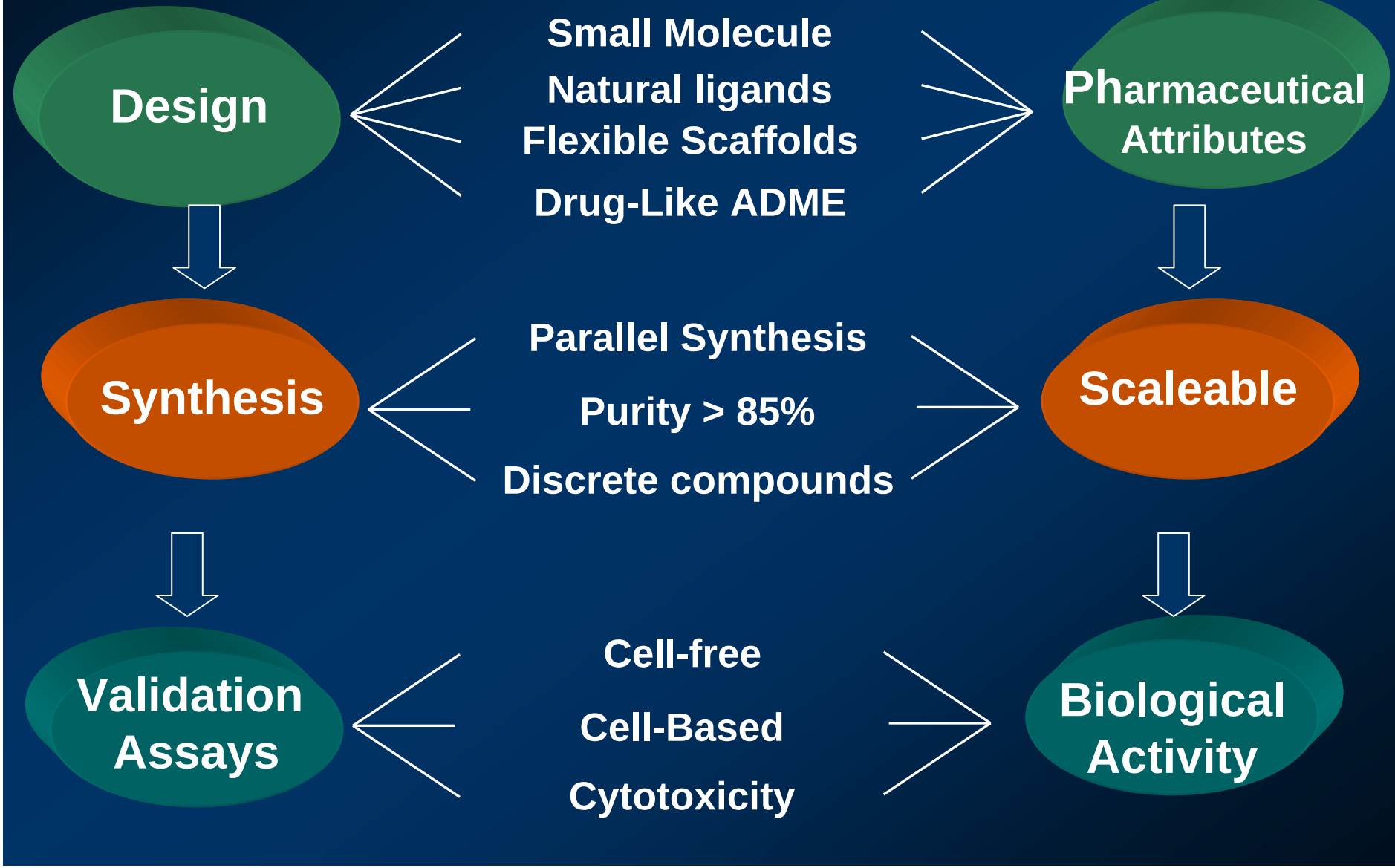
Combinatorial library based upon nucleotide scaffold as a strategy for drug discovery





Phosphate groups is key binding element in nucleotide – staphylococal nuclease interactions

Nucleotide library in drug discovery



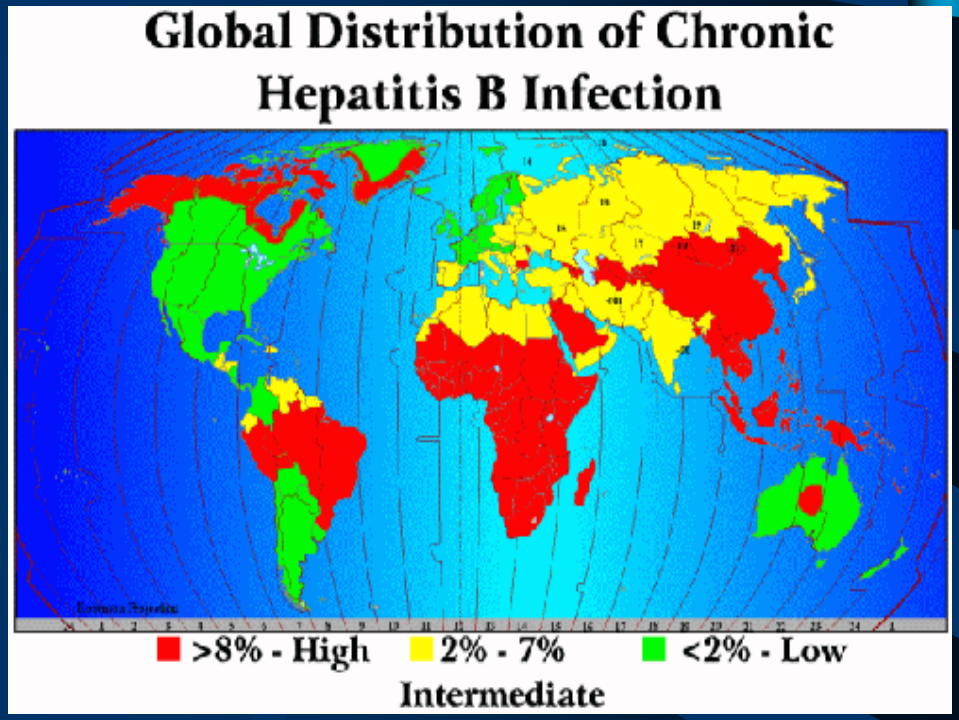
Validation of nucleotide library approach Discovery of anti-HBV agents

"Concerted efforts are urgently needed to define key steps in infection, replication, and assembly, as well as, the design and testing of new classes of curative HBV antivirals"…… …

National Institutes of Health (2002)

Global Crisis due to HBV Infection

- HBV causes both acute and chronic infection
  - ✓ 2 billion infected world-wide
  - ✓ About 350 million are chronically infected
  - ✓ 1 million deaths per year

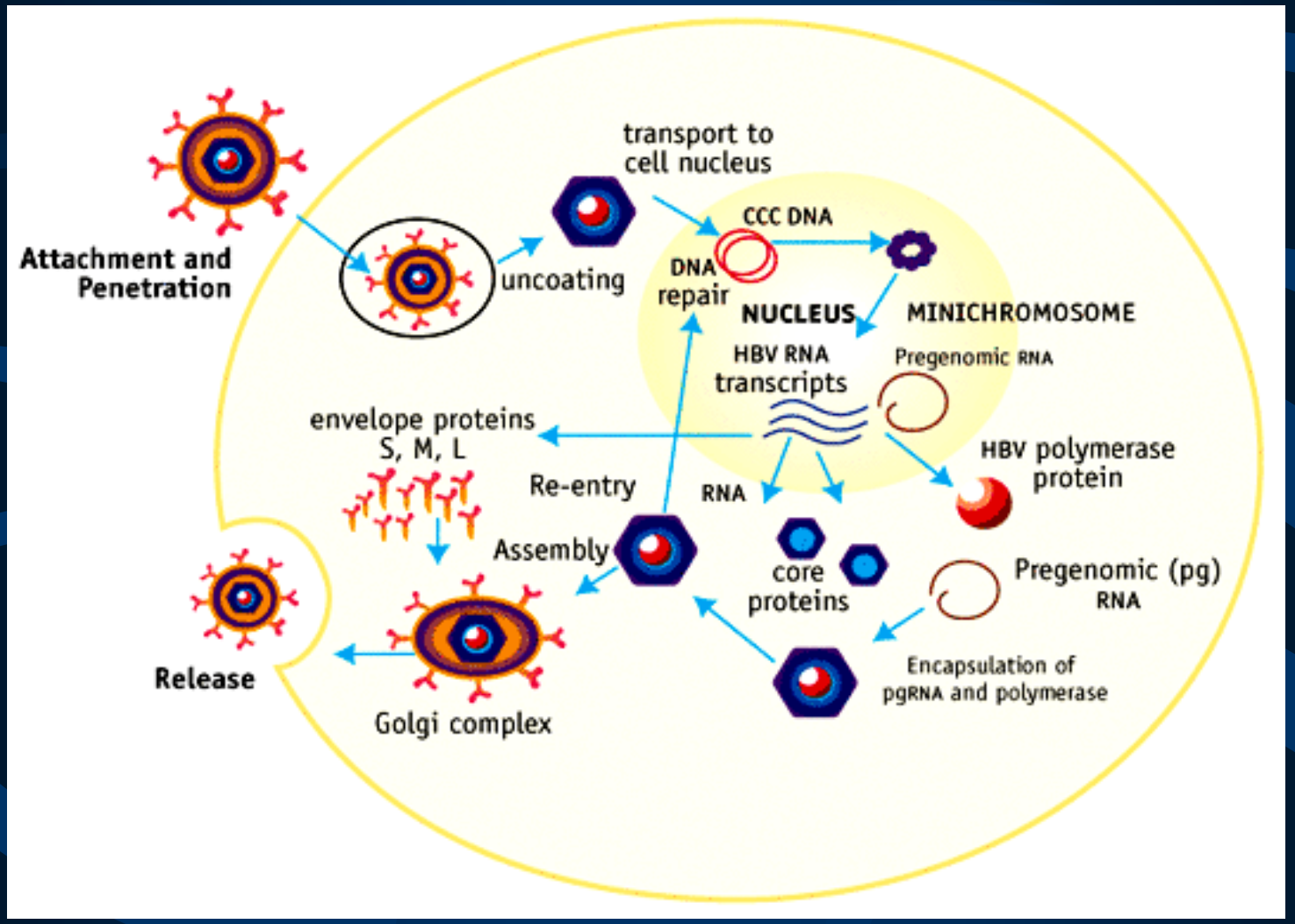


Transmision routes:

- ✓ Sexual intercourse
- ✓ Intravenous drug use
- ✓ Blood products

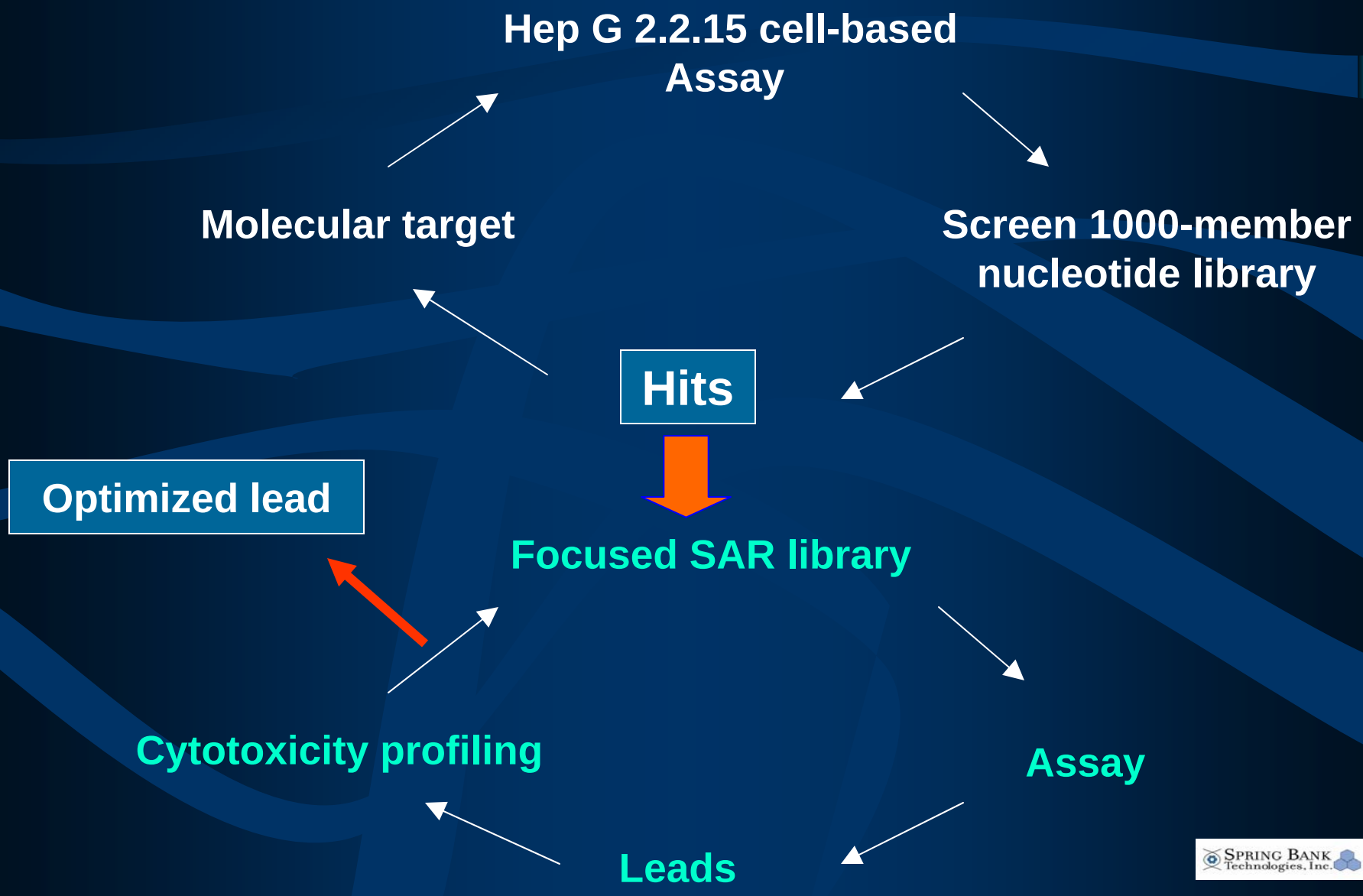
Spring Bank Technologies, Inc.

HBV Life Cycle



SPRING BANK Technologies, Inc.

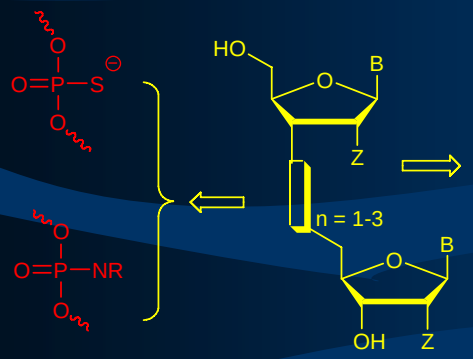
Phenotypic Approach to HBV Lead Discovery



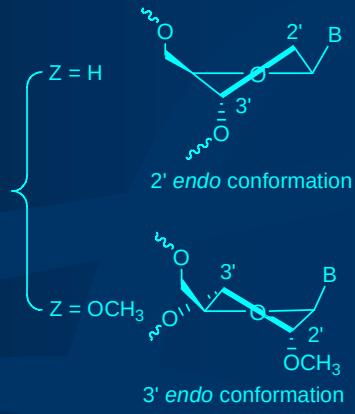
The library of nucleotides

A library of di-, and tri-nucleotides

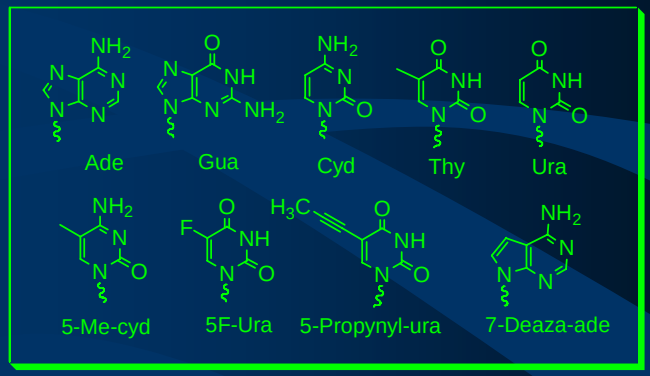
Linkage



Sugar conformation



Nucleobase



Antiviral Screening

Actives

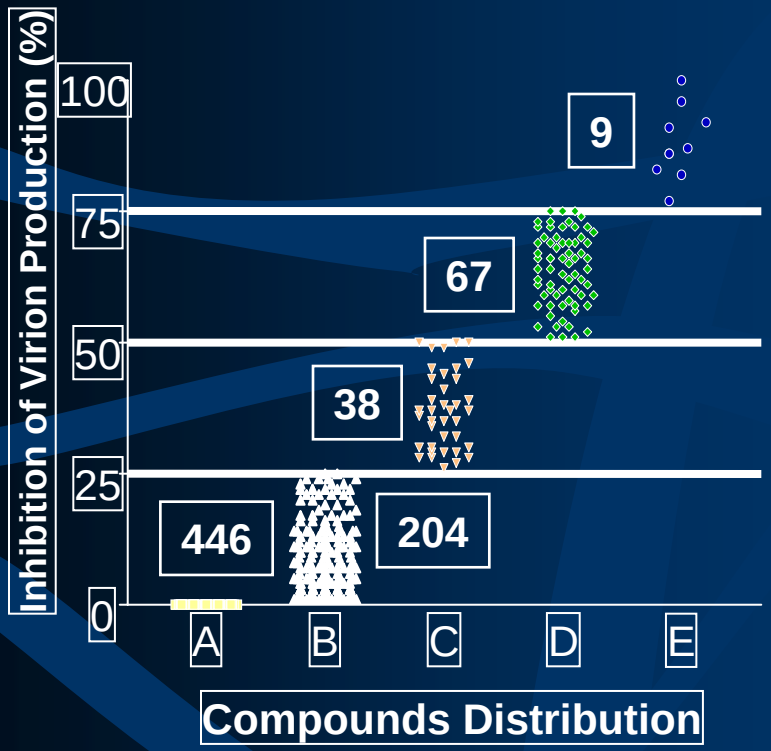
Lead Optimization

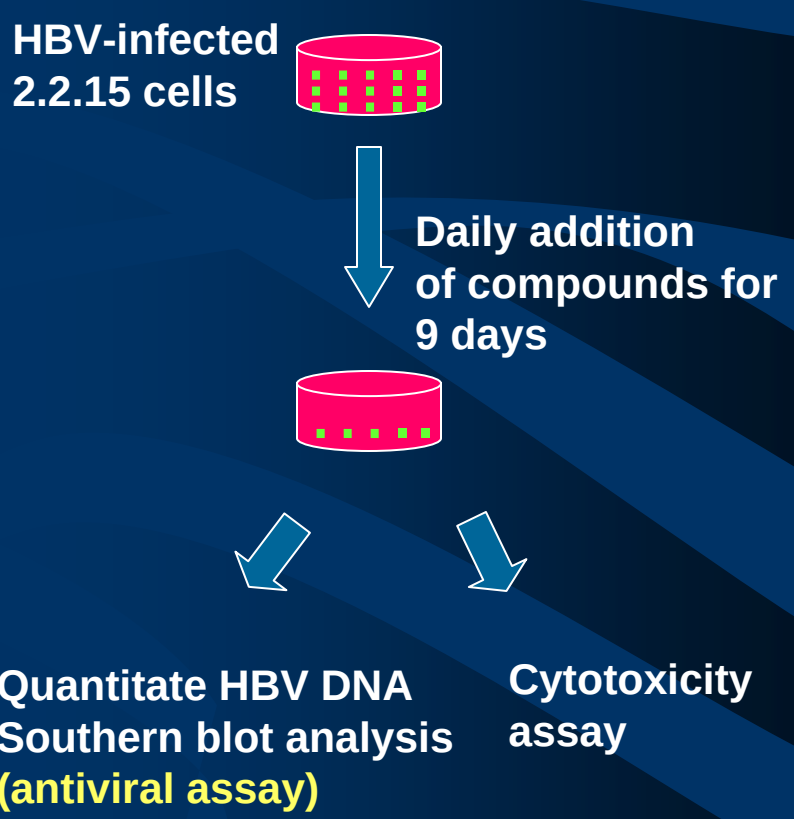
Lead

Spring Bank Technologies, Inc.

Lead discovery using cell-based antiviral assay

Distribution of actives





In collaboration with Dr. Brent Korba, Georgetown University

SPRING BANK Technologies, Inc.

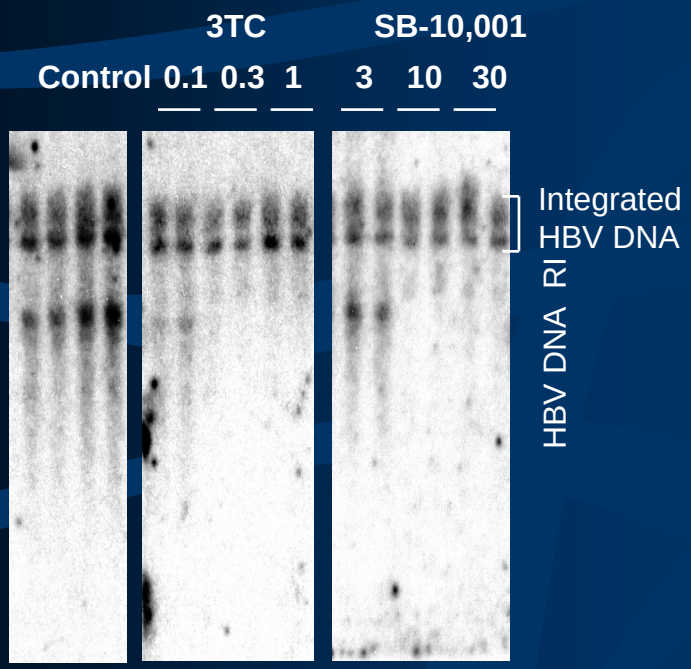
Lead Discovery Highlights - HBV Program

- ✓ Over 1400 compounds screened in assay
- ✓ Four potent compounds discovered following SAR
- ✓ **Novel Di-, and trinucleotide compounds**
- ✓ High safety index (CC_{50}/EC_{50} > 1000)
- ✓ Potency , EC_{50}, 0.3 micromolar, comparable to Adefovir

SB 9000 - a Novel Anti-HBV Nucleotide

SPRING BANK Technologies, Inc.

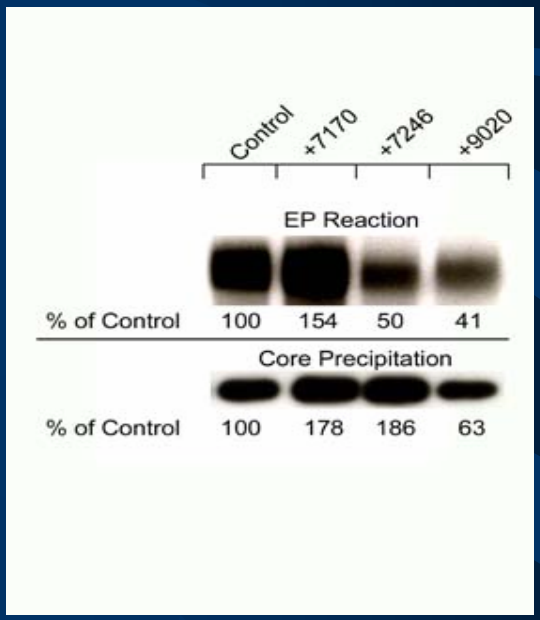
SB 9000 analogs are intracellular inhibitors of HBV replication



Southern blot analysis of HBV DNA after 14 days treatment

In collaboration with Dr. Brent Korba, Georgetown University

Di-, and tri-nucleotide compounds inhibit HBV Endogenous Polymerase



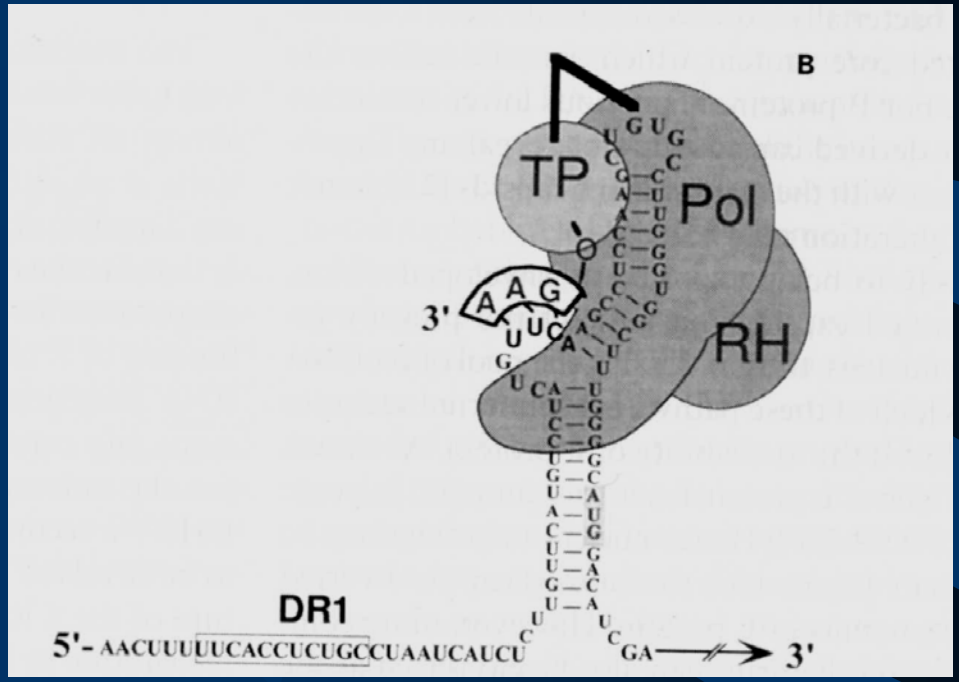
Huh 7 cells transfected with HBV DNA were treated with 10 uM of each compound for 72 h.

In collaboration with University of Texas, San Antonio

SPRING BANK Technologies, Inc.

Multiple mechanisms of action of SB 9000

- Inhibits HBV DNA synthesis
- Inhibits viral polymerase by a mechanism other than chain termination
- Inhibition of priming step during viral nucleic acid synthesis



SPRING BANK Technologies, Inc.

Anti-HBV profile of SB 9000

Combination with	SB 9000
3TC	Moderately synergistic
Adefovir	Additive to strong antagonist

No synergistic cytotoxicity observed

SB 9000 and analogs are potent inhibitors of resistant HBV mutants

HBV type	Compound EC_{90}	
	3TC	SB 9000
Wild type	0.6	9.0
M204 v	>100	9.8
M204i	>100	10
L180m	18	12

Cultures were treated for three days beginning 72 hours post-transfection, four replicates per concentration. S.D. not shown. Activity comparable to adefovir

In collaboration with Dr. Brent Korba, Georgetown University

SPRING BANK Technologies, Inc.

SB 9000 is a selective antiviral agent

	SB 10001 IC_{50} (uM)	SB 9000 IC_{50} (uM)
HBV	0.6 to 1.1	0.5 to 1.5
BVDV (NADL)	> 50	> 50
HCMV (AD169)	> 20	> 20
YFV	> 50	> 50
HSV (KOS)	> 20	> 20
HIV-1 (IIIb)	**> 2**	**> 2**

Collaborative study: Mark Wainberg (HIV), Brent Korba (HBV) and Viridae sciences (YFV)

SPRING BANK Technologies, Inc.

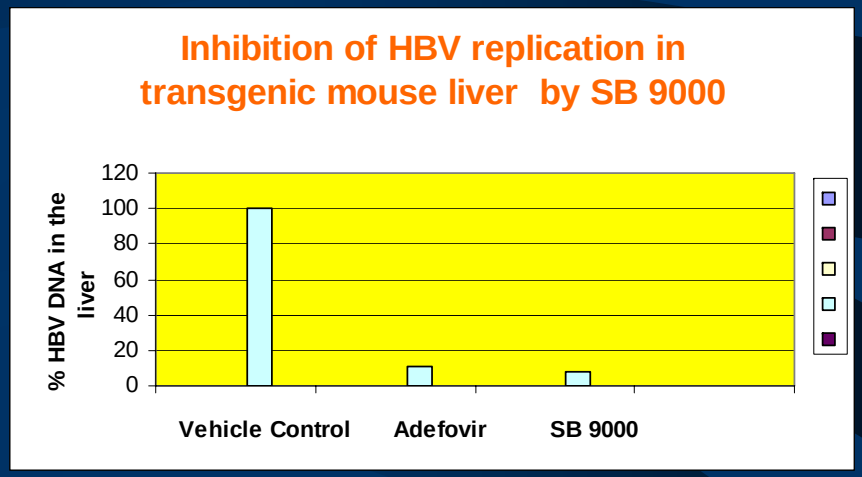
Efficacy Studies of SB 9000 in Animal Models of HBV

SPRING BANK Technologies, Inc.

Antiviral evaluation of SB 9000 in transgenic mouse model of HBV

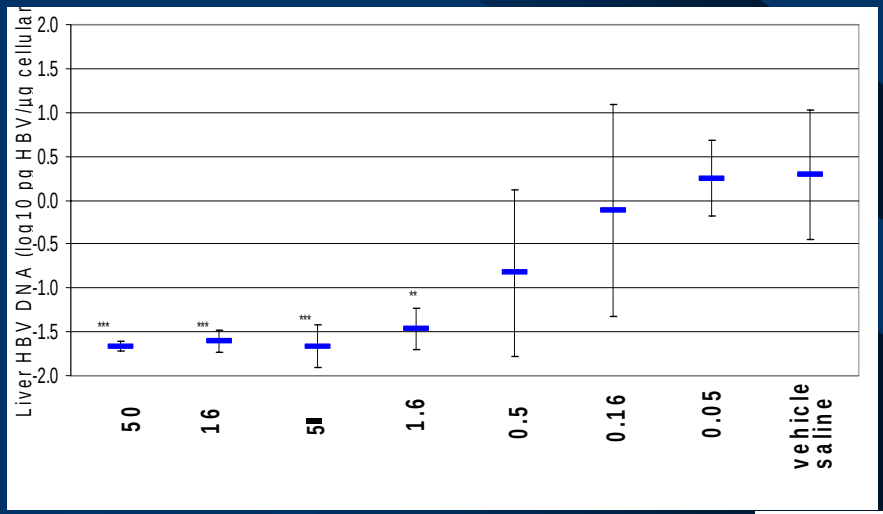
Initial high-dose study

- 14-day daily administration IP route
- 100 mg/Kg SB 9000, ADV 10 mg/Kg
- **End point**: reduction in Liver HBV DNA on day 14 - quantitative PCR and southern blot analysis



Dose-response study

- EC_{50} of SB 9000 is <1 mg/Kg
- More potent than adefovir

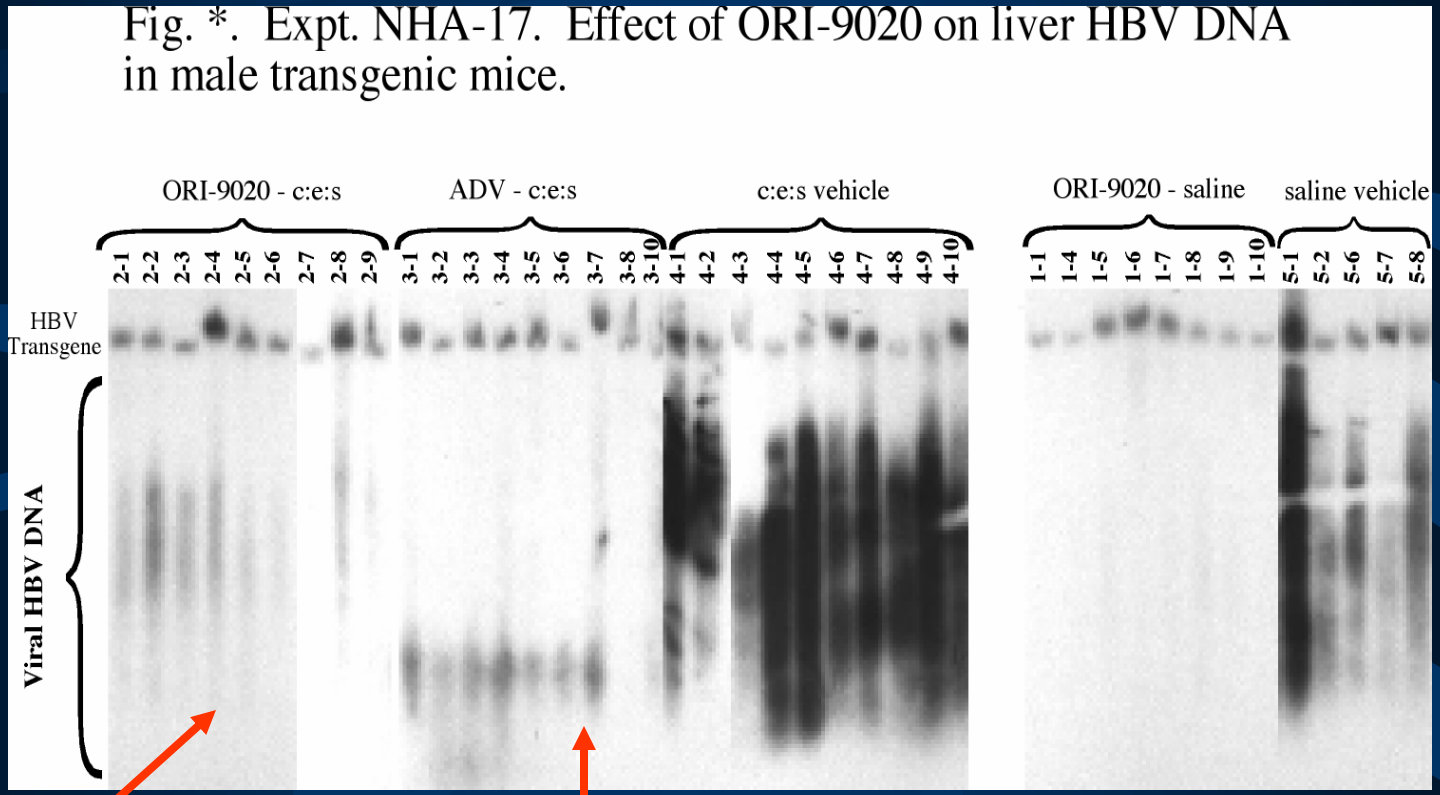


In collaboration with Dr. John Morrey, Utah State University

SPRING BANK Technologies, Inc.

SB 9000 shows strong anti-HBV activity in transgenic Mice model of HBV infection

Southern blot analysis of liver HBV DNA following 14-day treatment



Absence of Low MW HBV DNA species

SB 9000 (CES) *Adefovir* *CES (control)* *SB 9000 (saline)* *Saline (control)*

There was no toxicity associated with the administration of the drug

In collaboration with Dr. John Morrey, Utah State University

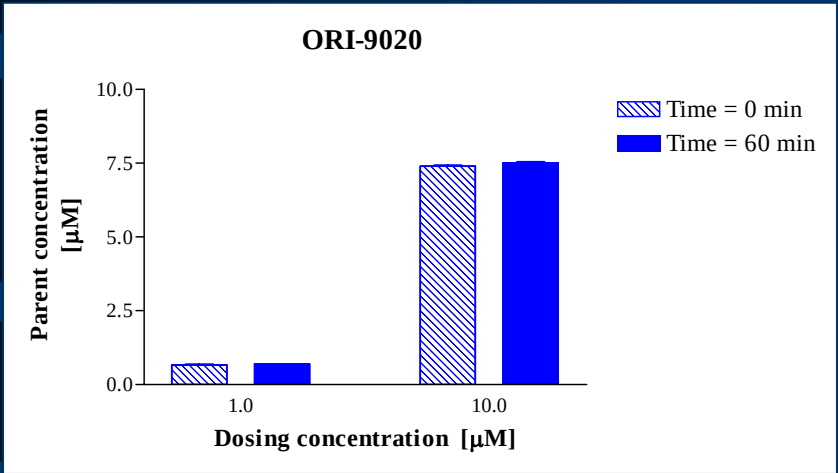
Spring Bank Technologies, Inc.

Drug Development

Spring Bank Technologies, Inc.

Pharmaceutical properties of SB 9000

- Metabolically stable in vitro and in vivo



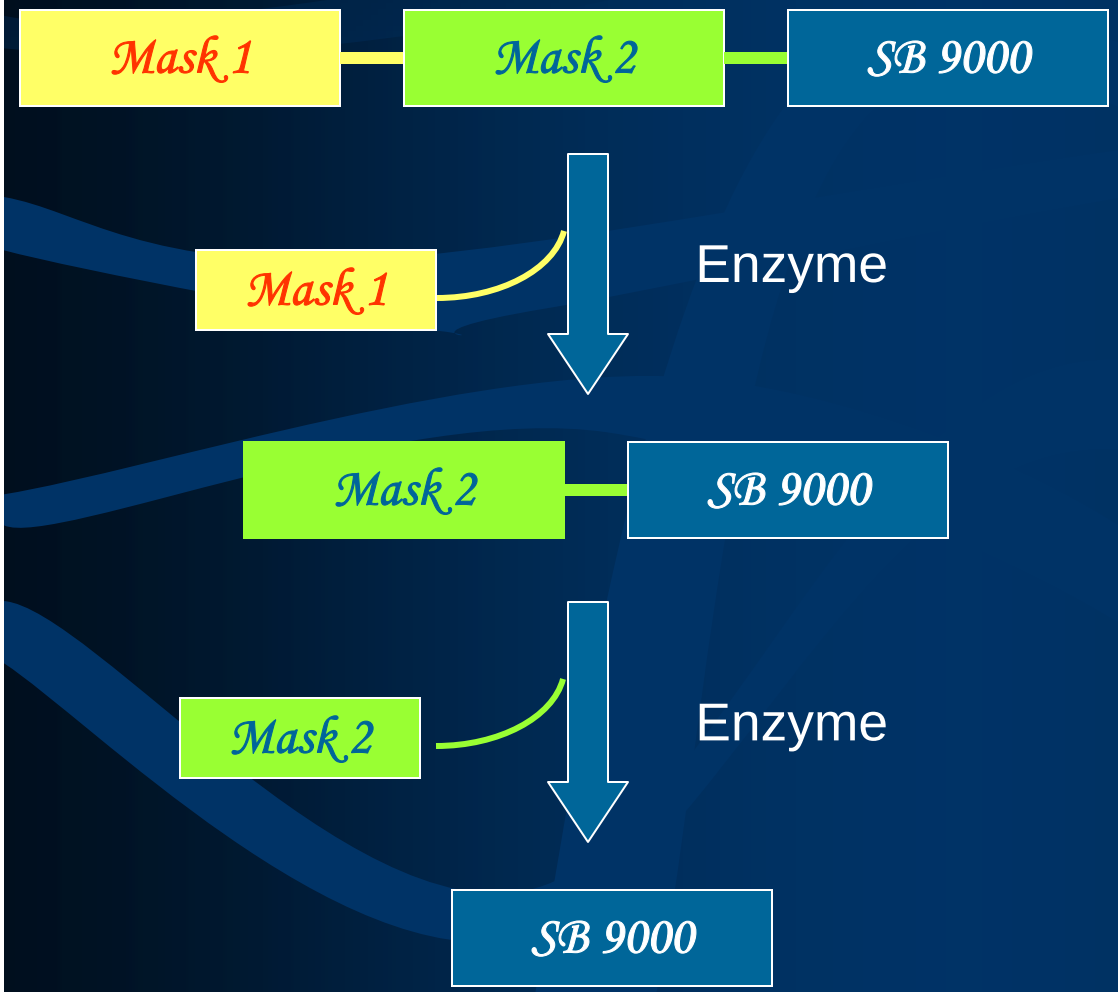
- Significant tissue disposition in liver

Issues

- Not orally bioavailable
- Not stable in gastric fluid
- Not much known about nucleotide drug transporters in GI tract

Spring Bank Technologies, Inc.

Tripartate prodrugs of SB 9000 for oral bioavailability



SB 9000 prodrugs

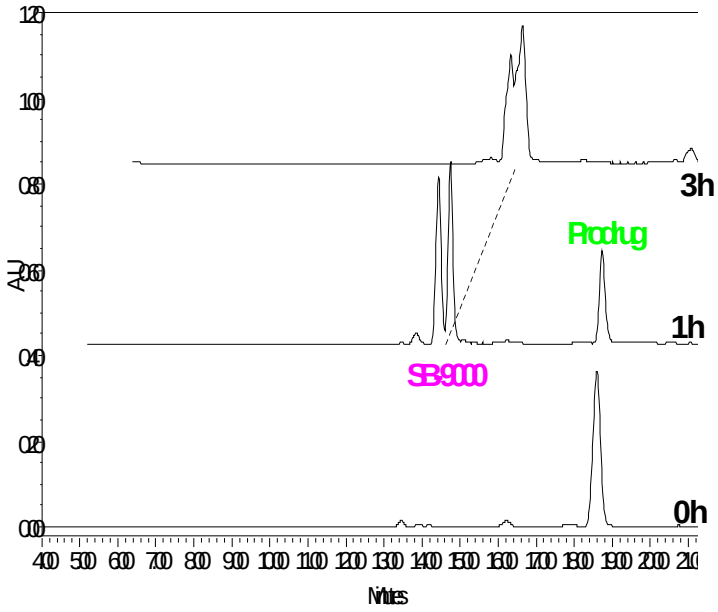
- Well-established drug regeneration pathway
- Stable in GI tract
- Properties suitable for Formulation
- High safety
- Orally bioavailable

SPRING BANK Technologies, Inc.

Characteristics of some SB 9000 prodrugs

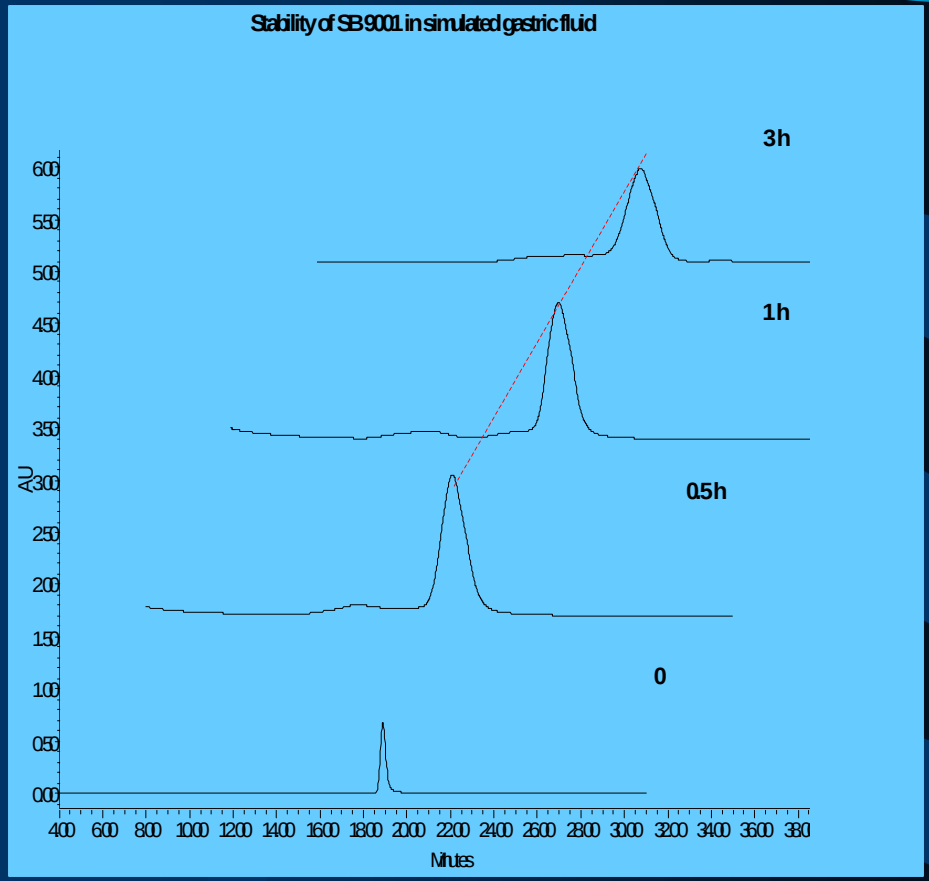
Serum conversion

HPLC profile depicting the kinetics of conversion of Prodrug to SB9000 in rabbit serum



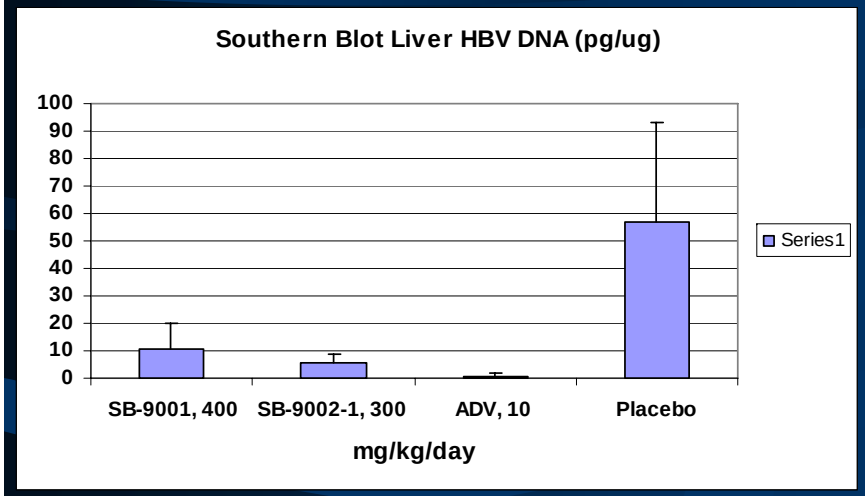
Prodrug ⟹ SB9000

Stability in gastric fluid



SPRING BANK Technologies, Inc.

Antiviral and safety studies of oral SB 9000 prodrugs in transgenic mice



Initial high-dose study

Animals: male and female transgenic mice (founder 1.3.32)

Placebo: 0.05 M citric acid, pH 2.0

Adefovir 10 mg/kg/day positive control

Prodrug	QPCR Liver HBV DNA pg/microg	Southern blot Liver HBVDNApg/microg
SB 9001 400 mg/Kg/day	24.3 ± 19	**10.5 ± 9.3****
SB 9002-1 300 mg/Kg/day	13.3 ± 12	**5.7 ± 3.2****
Placebo	65 ± 79	**57 ± 36**

Action Plan for development of SB 9000

IND-tox studies planned for 2007

IND 2008

Initiate clinical trials

SPRING BANK Technologies, Inc.

SB 9000 program summary

- Potent, safe, selective “first in class” anti-HBV agent with novel mechanism of action
- Synergistic with other antivirals and active against 3TC-resistant strains
- An orally bioavailable prodrug has been developed that is an active anti-HBV agent in vivo
- Nucleotide Discovery concept broadly applicable to other disease targets

Spring Bank Technologies, Inc.

Acknowledgments

In vitro studies

Dr. Brent Korba, Georgetown University

Professor Robert Lanford, University of Texas

In vivo studies

Professor John Morrey, Utah State University

Dr.Bud Tennant, Cornell University

- Biology and chemistry team at Origenix Technologies
- Spring Bank technologies, scientific team

S. Padmanabhan, Ph.D.

John Coughlin,M.A.

G. Zhang, Ph.D.

C. Kirk, Ph.D.

SPRING BANK Technologies, Inc.

We Thank

NIH, NIAID for the Grant award

UO1 AI 058270 [2003-2008]

Special thanks to Dr. Diana Berard, Program Officer, NIAID

R. P. Iyer, Ph.D.
Spring Bank Technologies, Inc.,
113 Cedar Street, Milford, MA 01757, USA.
kiyer@springbanktech.com
508-473-5993